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Meeting Report

Cell death people share their knowledge on killing at Nobel Forum

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Presented at the first Cell Death Network mini-symposium at Nobel Forum, Karolinska Institutet, Sweden on 14 April 2008.

The Network on the Mechanisms and Biomedical Implications of Cell Death or simply the 'Cell Death Network' (www.celldeathnetwork.com) was proud to organize its first symposium on 14 April 2008, in the prestigious Nobel Forum lecture hall at Karolinska Institutet, Stockholm. Cell death is a very broad notion, a process involved in a wide variety of biological states. Cell death, in the form of apoptosis, necrosis or autophagy, is involved not only in diseases such as cancer and allergy, but also in the normal process of tissue renewal and development. Thus, the aim of this meeting was to bring researchers from different fields, with a common interest in cell death, together to discuss cell death from different angles. The program of the meeting covered topics within immunology, neurology and oncology as well as growth and aging. Both local and invited speakers presented their latest unpublished findings leading to vivid discussions, which continued also during lunch and coffee breaks.

In his introductory remark, Sten Orrenius, the senior advisor of the Cell Death Network, put forward advances made in the field of cell death research during the last years. Although several mechanisms have been resolved, many questions still remain and more arise as new theories emerge. Sten Orrenius also pointed out that numerous compounds/drugs aiming at the cell death machinery have been elaborated in the past few years. Importantly, several of them have already entered the clinical trial phase, with the hope of beneficial effects in various diseases. This latter point emphasizes the biomedical implication of cell death in human disorders and the therapeutic potential of targeting it.

Molecular Mechanism of Cell Death

Several of the talks delivered throughout the day dealt with the different modes of cell death and its biological importance.

Gerry Melino discussed in his talk two interesting stories about p63. Besides the fact that p63 is the oldest member in the p53 family, p63 has many isoforms due to different translation starting points and alternative C-terminal splice sites. Melino and co-workers have shown that mutations in the SAM domain are responsible for the conformational change that is believed to be the cause of the extrodactyly ectodermal dysplasia and cleft lip/palate syndrome. The second part gave a new insight into the world of p63, as it was suggested that p63, besides its involvement in epidermal development and differentiation, is involved in the conservation of epithelial stem cells.

The group of Aleksandra Trifunovic has developed a mouse model that provides the first experimental evidence for a causative link between mitochondrial (mtDNA) mutations and aging phenotypes in mammals. The mtDNA mutator mice were engineered to have a defect in the proofreading function of mitochondrial DNA polymerase (*Polg*), leading to the progressive, random accumulation of mtDNA mutations during the course of mitochondrial biogenesis. The mtDNA mutator mice display a completely normal phenotype at birth but subsequently acquire many features of premature aging. The increase in somatic mtDNA mutations is associated with reduced life span and premature onset of aging-related phenotypes. Aleksandra Trifunovic reported that the mtDNA mutator mice develop a hearing loss due to progressive degeneration of both peripheral and central auditory neurons. TUNEL-positive neurons, as well as an increase in the number of neurons expressing activated caspase-3 and activated caspase-7, indicate that this cell loss probably occurs as a consequence of apoptosis. High levels of apoptotic cells were also detected in both proliferating (thymus, intestine, testis) and post-mitotic (skeletal muscle, brain) tissues in two separate mtDNA mutator strains.

Proteasome inhibitors (PIs), a novel class of anti-cancer drugs, have recently been introduced in the clinic for the treatment of multiple myeloma and are currently in clinical trials for the treatment of childhood cancers. The tumor selectivity and low toxicity of PIs are surprising, given the crucial role of the ubiquitin/proteasome system (UPS) in a multitude of vital cellular processes. Addressing the risk of somatic cells during PI treatment, Farazat Zaman and co-workers used an array of model systems including transgenic GFP reporter mouse (to monitor UPS *in vivo*),

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fetal metatarsal organ cultures and chondrogenic cell lines. They found that systemic administration of PIs caused significant impairment of the UPS, accompanied by induction of p53- and AIF-mediated apoptosis, specifically in stem cell-like chondrocytes of the growth plate but not in other tissues, and resulted in severe growth retardation.

The UPS and its role in protein toxic stress-induced cell death were further discussed by Nico Dantuma. For this purpose, his group has developed a number of fluorescent reporters for the UPS. These reporters are based on green fluorescent protein and other fluorescent proteins. Ubiquitin tagged with various fluorescent proteins has also been used to follow ubiquitin and ubiquitylation in living cells. With these fusions, they can follow different pools of ubiquitin in dying cells. It appears that ubiquitylation is used not only for protein degradation but also for membrane trafficking, activation of proteins, DNA repair and transcriptional regulation.

Boris Zhivotovsky's presentation focused on analysis of various modes of cell death induced by DNA damage. It appears that the response depends not only on the type of treatment and dose used, but also on the molecular profile of the cell. In fact, activation of p53 by cellular stress may lead to either cell cycle arrest or apoptotic cell death. Restrictions in the ability of a cell to halt the cell cycle might, in turn, cause mitotic catastrophe, a delayed type of cell death with distinct morphological features. Experiments performed on ovarian carcinoma cells revealed that both functional p53 and caspase-2 were required for apoptotic response. However, in the absence of functional p53, similar treatment resulted in caspase-2-independent mitotic catastrophe followed by necrosis. Hence, p53 appeared to act as a switch between apoptosis and mitotic catastrophe. Boris Zhivotovsky proposed that death-associated mitotic catastrophe is not a separate mode of cell death, but rather a process ('pre-stage') preceding cell death, which can occur through necrosis or apoptosis.

Dan Grandér presented data on the mechanism of glucocorticoid (GC)-induced cell death in lymphoid leukemic cells. In their studies, the group has found GCs to induce cell death with autophagic features in both cell lines and primary leukemic cells. Interestingly, the autophagy preceded other cell death markers, and blocking autophagy (chemically or genetically) blocked the ability of the GC to induce cell death. This indicates that GC-induced autophagy in leukemic cells is necessary to trigger apoptosis and death. The group has also determined the induction of the promyelocytic leukemia protein (PML) in susceptible cells and the subsequent PML-mediated inactivation of Akt/PKB through dephosphorylation as a key event in GC-induced autophagy and cell death. Overall, these findings may help resolve the important clinical issue of GC resistance and aid in sensitization to GC therapy.

Apoptosis as the End Point of Targeting Therapy

Targeted therapy with small molecules has proven quite successful in the treatment of several malignancies and is often considered the future in cancer treatment.

Owing to its frequency in tumors, mutant p53 is an attractive target for novel cancer therapy. Klas Wiman and co-workers have screened for small molecules that preferentially target mutant p53-expressing human tumor cells and identified PRIMA-1 several years ago. This compound was shown to restore wild-type conformation to mutant p53, induce mutant p53-dependent apoptosis in human tumor cells and inhibit xenograft tumor growth in mice. The Wiman group has shown that PRIMA-1 and its structural analog PRIMA-1^{MET} induce expression of PUMA and Bax, two pro-apoptotic p53 targets, as well as activation of several caspases in a mutant p53-dependent manner. A selected PRIMA-1 analog will be tested in clinical phase-I trials this year.

Marie Arsenian Henriksson discussed a similar strategy for the identification of small molecules with potential use for cancer therapy. Her group has identified Myc pathway Response Agents (MYRAs) that induce apoptosis in a c-Myc- and MYCN-dependent manner and inhibit Myc-driven cellular transformation. The MYC network of transcriptional regulators plays a key role in the regulation of cell growth, apoptosis and differentiation, and MYC is activated in a number of different tumors. The frequent amplification of the *MYCN* gene, an established causal link between the malignant phenotype and the resistance to treatment, along with its restricted expression in normal tissues, makes MYCN an attractive therapeutic target. MYRA-A interferes with the DNA-binding activity of Myc family proteins, whereas MYRA-B induces Myc-dependent apoptosis without affecting Myc transactivation or Myc/Max DNA binding. A third compound, NSC308848, also induced apoptosis in Myc- and MYCN-overexpressing cells and inhibited Myc-induced cellular transformation. However, in contrast to the MYRAs, NSC308848 treatment decreased Myc protein levels and also gave rise to inhibitory effects on transcription factors other than Myc. At present, the molecular mechanism of 10 novel candidate compounds that induced apoptosis in a MYCN-dependent manner are analyzed in more detail.

Cellular receptors, such as VEGFR and EGFR, have been known to stimulate cell growth upon activation after binding to their ligand. Here Patrick Mehlen has shown a new kind of membrane receptors, the so-called dependence receptors, which in the absence of binding to their ligand lead to the activation of the cell death machinery. Such receptors are the Netrin-1 receptors, DCC and UNC5H, which genes are found to be frequently deleted or mutated in human cancers. Using these new findings and the fact that Netrin-1 is often overexpressed in metastatic breast tumor cells, thus promoting their survival, Mehlen and co-workers are now developing drugs to induce apoptosis by preventing Netrin-1 from binding to its receptors.

Also, Tomas Ekström presented his most recent progression in the development of a new therapeutic approach to treat malignant glioma. The technique is based on intercellular exchange where neural progenitor cells act as carriers of the drug. The method takes advantage of the gap-junction communication within a cell population, which allows drug transfer from the neural progenitor into the surrounding target cells.

Uncontrolled cell proliferation and survival is not solely a phenomenon associated with tumor formation, but it is also of major importance in allergy and other inflammatory disorders. The main culprit in these conditions is the mast cells. Thus, one possible therapeutic intervention could be to induce mast

cell apoptosis, thereby reducing the number of tissue mast cells, which should decrease symptoms. Induction of mast cell apoptosis would also be plausible in mastocytosis, a myeloproliferative disease without curative treatment. By a genetic approach, Gunnar Nilsson and co-workers have investigated the mechanisms involved in regulating mast cell longevity and survival in health and disease, and identified Bcl-2-family members as crucial regulators of mast cell survival and apoptosis. Bcl-2 and Bcl-XL are important for the late phase of mast cell development, whereas A1/Bfl-1 is crucial for activation-induced mast cell survival upon allergic reaction. Small molecule inhibitors acting on the prosurvival Bcl-2-family members induce mast cell apoptosis and also prohibit activation-induced mast cell survival. Future research will provide evidence if this knowledge can be transferred into new therapies to treat mast cell-associated diseases.

Concluding Remarks

This meeting gave a succinct update of the wide field of cell death and highlighted the importance of this process in biology and medicine. Despite the thousands of articles published on the topic, many aspects still remain to be investigated. Besides presenting promising development in the use and understanding of cell death with the aim of curing diseases, this meeting showed the importance of cross talk between different research fields, which, despite the fact that they focus their work on different projects at first glance, still share the basic mechanism of cell death.

As organizers, we are very pleased with the first Cell Death Network mini-symposium at Nobel Forum, the discussion it generated between the juniors and the more senior participants, the establishment of collaborative work or, more simplistically, the exchange between fields of research of methods to study cell death. Importantly, we are also looking forward to next year for the second round.

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